

Attorney Docket No.: PTQ-0027  
Inventors: Van Eyk et al.  
Serial No.: 09/115,589  
Filing Date: July 15, 1998  
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In the Specification:

Please replace the paragraph at page 23, lines 1-9 with the following:

E1 -- For example, when qualitatively characterizing different myofilament proteins and/or modification products present in the biological sample, antibodies can be used which differentially recognize epitopes present in the various modification products. Using a label that has a measurable moiety attached to it (e.g.,  $\beta$ -galactosidase), a profile or "fingerprint" of the proteins and modification products can be obtained. This profile, which is expected to include, for example, characteristic ratios of various proteins and/or fragments from the same (e.g., cardiac TnI residues 1 to 193 of SEQ ID NO:8 vs. cardiac TnI residues 63 to 193 of SEQ ID NO:8) or from different (e.g., TnI vs. myosin light chain I) proteins, can then be associated with a level (i.e., extent) or type of myocardial damage.--

Please replace the paragraph at page 23, lines 10-18 with the following:

E2 -- Different myofilament proteins and/or modification products present in the biological sample can also be quantitatively characterized (e.g., compared to a standard). For example, levels of different troponin I modification products (e.g., a cardiac

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troponin I fragment consisting of amino acids 1 to 193 of SEQ ID NO:8) can be compared to one another, or to levels of the intact troponin I protein, and this pattern of protein levels can be associated with a level (i.e., extent) or type of myocardial damage. Levels of myofilament proteins and/or modification products can be detected using for example quantifiable labels (e.g. antibodies labeled with an enzyme, the activity of which can be measured and correlated with levels of antibody binding), as are known in the art, which specifically bind to the proteins and/or modification products. --

Please replace the paragraph at page 23, lines 19-21 with the following:

-- In one embodiment, the method of the invention is used to diagnose mild ischemia by detecting the presence of skeletal or cardiac troponin I fragment (e.g., cardiac TnI residues 1 to 193 of SEQ ID NO:8) and comparing the levels of this fragment to the levels of intact troponin I. --

Please replace the paragraph at page 24, lines 1-11 with the following:

For example, we describe herein that the extent and type of modification to TnI (amino acid residue 1 to 210 of SEQ ID NO:8) change depending on whether mild or severe ischemic damage has

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E4

occurred. With mild ischemia and/or ischemia/reperfusion, TnI is specifically degraded, yielding a fragment with apparent molecular weight of 22 kDa by SDS-PAGE, corresponding to amino acid residues 1 to 193 of SEQ ID NO:8, due to proteolysis of 16 amino acid residues from the C-terminus of TnI. In addition (or shortly thereafter), TnI 1 to 193 of SEQ ID NO:8 forms covalent complexes with TnC or TnT (32 kDa by SDS-PAGE). Later, with increasing severity of ischemic and/or ischemic/reperfusion damage, TnI is further degraded, yielding smaller fragments TnI 63 to 193 of SEQ ID NO:8 and 73 to 193 of SEQ ID NO:8 (16 and 15 kDa by SDS-PAGE). Therefore, if a profile from a biological sample shows only a 22 kDa TnI protein fragment, rather than both a 22 kDa and a 16 kDa TnI fragment, the indication is that mild/reversible rather than severe/irreversible damage has occurred. --

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Please replace the paragraph at page 24, lines 12-18 as follows:

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E5

-- Different myofilament proteins are more or less susceptible to modification depending on the extent of ischemic or ischemic/reperfusion injury that has occurred. Thus, the appearance of a certain modification to a specific proteins can be used as a marker/index for extent of muscle damage. For example, MLC1 degradation (residues 20-199 of SWISS-PROT Accession No.

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P17209 (<http://www.expasy.ch/cgi-bin/sprot-search-de>) occurs only with very severe ischemia in the myocardium. Therefore, if one detects this smaller fragment of MLC1 in a biological sample, it is an indication that the myocardium is severely and possibly irreversibly damaged.--

Please replace the paragraph at page 24, line 26 as follows:

E6 -- 1. TnI degradation product residues 1 to 193 of SEQ ID NO:8 and loss of  $\alpha$ -actinin. --

Please replace the paragraph at page 24, lines 27-29 as follows:

E7 --2. TnI or TnI 1 to 193 of SEQ ID NO:8 covalent complex formation. (As proteolysis and covalent complex formation may occur very rapidly the two species may thus be indistinguishable from one another.)

Please replace the paragraph at page 25, line 1 as follows:

E8 --3. TnI further degraded (Residues 63 to 193 of SEQ DI NO:8). --

Please replace the paragraph at page 25, line 2 as follows:

E9 --4. TnI further degraded (Residues 73 to 193 of SEQ DI NO:8). --

#### REMARKS

With respect to troponin I residues or fragments disclosed at pages 23-25 of the specification, Applicants have amended the